Research Article

Localized micronutrient patches induce lateral root foraging and chemotropism in *Nicotiana attenuata*

Running Title: Directed root nutrient foraging in nature

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ABSTRACT

Nutrients are distributed unevenly in the soil. Phenotypic plasticity in root growth and proliferation may enable plants to cope with this variation and effectively forage for essential nutrients. However, how micronutrients shape root architecture of plants in their natural environments is poorly understood. We employed a combination of field and laboratory-based assays to determine the capacity of *Nicotiana attenuata* to direct root growth towards localized nutrient patches in its native environment. Plants growing in nature displayed a particular root phenotype consisting of a single primary root and a few long, shallow lateral roots. Analysis of bulk soil surrounding the lateral roots revealed a strong positive correlation between lateral root placement and micronutrient gradients, including copper, iron and zinc. In laboratory assays, the application of localized micronutrient salts close to lateral root tips led to roots bending in the direction of copper and iron. This form of chemotropism was absent in ethylene and jasmonic acid deficient lines, suggesting that it is controlled in part by these two hormones. This work demonstrates that directed root growth underlies foraging behavior, and suggests that chemotropism and micronutrient-guided root placement are important factors that shape root architecture in nature.

INTRODUCTION

Mineral nutrients vary in abundance in the soil in both space and time. As a possible consequence of this variability, plant roots display remarkable phenotypic plasticity in response to changes in nutrient concentrations. It is generally assumed that plants optimize root architecture to exploit nutrient rich patches and optimize nutrient uptake (Hodge 2004; Gruber et al. 2013). The spatial arrangement of roots, termed root system architecture (RSA), is a strong determinant of nutrient and water uptake efficiency and strongly influences plant growth and fitness (Fitter et al. 2002).

Nutrients shape RSA by altering the density, length, diameter or angle of roots and root hairs (Giehl and von Wirén 2014). Under homogenous phosphorus limitation, for example, roots exhibit a shallow root architecture that results from the inhibition of primary root (PR) elongation and an increase in lateral root (LR) elongation (Williamson et al. 2001; Sánchez-Calderón et al. 2005). This architectural change is thought to increase the plant's ability to effectively forage within phosphorous-enriched top soils (Rubio et al. 2003; Zhu et al. 2005). Mild N and P deficiency both lead to an increase in root-to-shoot ratios (Cakmak et al. 1994; Marschner et al. 1996) through stimulation in both PR and LR growth (Linkohr et al. 2002). However, under severe nitrogen limitation, lateral root formation is completely absent, demonstrating that plants require a baseline level of nitrogen to actively maintain growth (Krouk et al. 2010). Localized nutrient patches in nutrient-limited soils are also known to change RSA (Forde and Lorenzo 2001; López-Bucio et al. 2003; Malamy 2005; Osmont et al. 2007). In *Arabidopsis thaliana*, a localized supply of phosphate or nitrate increases LR elongation (Linkohr et al. 2002; Remans et al. 2006), while ammonium triggers LR branching (Lima et al. 2010). More recently, the localized supply of the micronutrient iron was shown to lead to a strong enhancement in LR emergence and elongation without having a differential effect on LR number (Giehl et al. 2012). Despite these findings, little is known about how micronutrients shape RSA in plants growing in their natural environments.

Exploring the soil for water and nutrients and providing anchorage may also rely on the ability of roots to adapt their growth towards or away from certain stimuli. Directed growth movements (also called tropisms) have been well established for roots with respect to gravity, light, water, mechanical stimuli, temperature, and electric or magnetic fields (Esmon et al. 2005), and can contribute significantly to the plant's survival and reproduction (Monshausen and Gilroy 2009). An unequal distribution of nutrients in the soil has also been thought to furnish opportunity for the development of chemical response (chemotropism) as a biological adaptation (Rosen 1962);

however, to date, the act of "foraging" has been investigated in the context of root placement or proliferation within high nutrient zones. Positive chemotropism has not been demonstrated for roots.

Simple root architecture traits including root placement, branching pattern, length, orientation, angle, and diameter are developmentally controlled by the same interacting genetic pathways that also modulate growth and developmental responses to nutrient cues (Jung and McCouch 2013). To date, the vast majority of research elucidating the genetic and hormonal pathways involved in RSA modifications induced by macronutrients, particularly nitrate, has focused on the phytohormone auxin (Péret et al. 2009; Giehl and von Wirén 2014). For example, genes that link localized nutrients and root growth in *A. thaliana* include the auxin receptor-miRNA module AFB3-miR393 and its downstream target *NAC4*, which control root system architecture in response to nitrate (Vidal et al. 2010; Vidal et al. 2013). The expression of a nitrate transporter (*NRT1.1*) and its downstream MADS-box transcription factor (*ANR1*) are also known to mediate local signaling to control selective root placement in the presence of high nitrate patches (Zhang and Forde 1998; Linkohr et al. 2002; Remans et al. 2006). Finally, hormones like ethylene and jasmonic acid also confer root developmental and behavioral changes related to RSA (Jung and McCouch 2013).

Here we explored changes to root ecophysiology and chemotropism induced by heterogeneous nutrient distribution in coyote tobacco, *Nicotiana attenuata* Torr. Ex Wats (Solanaceae) plants growing within their natural environment. As an annual plant that synchronizes its growth with post-fire environments in the Great Basin Desert of the southwestern USA, *N. attenuata* is thought to effectively exploit ephemeral and highly heterogeneous nutrient-rich habitats that are created by fires (Lynds and Baldwin 1998). To determine whether *N. attenuata* roots are able to direct their growth towards nutrient rich patches, experiments were designed to associate changes in root system architecture with soil nutrient gradients in natural *N. attenuata* populations. The acquired knowledge was then used to gain mechanistic insights into the regulation of specific root behaviors in laboratory assays. Together, these results reveal that selective placement and growth plasticity in *N. attenuata* roots, including chemotropism, enable the plant to match its root architecture to the abiotic environment.

RESULTS

Comparison of natural and glasshouse *N. attenuata* root systems

In contrast to the root systems of glasshouse-grown *N. attenuata* plants, which consisted of multiple, twisted primary and lateral roots (Figure 1A), plants excavated from naturally growing populations in Utah contained a single primary root and few, well-developed lateral roots extending outward into the shallow layers of the soil. This phenotype appears to be independent of plant ontogeny (Figure 1A). Of the 20 plants excavated from natural populations, 14 plants produced between 1 and 5 lateral roots (Figure 1B). In total, 18 plants (90%) had few, well-developed lateral roots which remained within shallow layers of soil (first 5 cm). In some cases, these roots extended horizontally for up to 40 cm (Figure 1C). Common root morphologies also included root proliferation and the formation of first order lateral roots within dense soil substrates (Figure 1C).

Lateral root placement in nature corresponds to high soil micronutrient concentration

To test whether specific nutrient gradients are responsible for generating LR elongation phenotypes in natural soils, we harvested bulk soil surrounding foraging LRs of naturally occurring *N. attenuata* plants. We found that LR placement was associated with a higher concentration of soil micronutrient levels relative to control, root-free transects (Figure 2A). Principle component analysis revealed a highly significant positive correlation between the concentrations of micronutrients Zn, Cu and Fe and sampling distance along foraging lateral roots (Figure 2A; Zn, P=2.00e^{-04}; Cu, P=0.008; Fe, P=0.0038). A significant negative correlation between sampling distance and K concentrations (Figure 2A; K, P=0.0177) was also observed—there was a tendency for the concentration of this element to decrease along roots relative to respective control transects (Figure 2D). Independent analyses of Zn, Cu and Fe concentrations between control and root transects revealed that these micronutrients were higher along foraging roots relative to the control transect (Figure 2E–G). Interestingly, soil samples containing the highest Zn, Cu and Fe concentrations (0.49 mg/kg, 0.67 mg/kg. and 7.05 mg/kg, respectively) were associated with pronounced changes in root morphology and structure, including visible increases in proliferation and the formation of first order lateral roots (as shown in Figure 1C). By contrast, no correlation was observed between N and Mn and sampling distance (Figure 2B, H). P levels were found to be significantly higher overall when control and root transect were compared (Figure 2C; P=0.022).

Localized micronutrient patches induce lateral root foraging and chemotropism in a concentration-specific manner

An agar coring technique (Figure 3A) was developed to examine changes in root placement and foraging responses in the presence of localized nutrient patches to better understand the connection between micronutrients and LR growth. Based on our field data that suggested a strong influence of Zn, Fe and Cu, we tested root growth responses to localized $CuFe_4O_4Zn$ patches. Under homogenously low micronutrient conditions, LRs displayed no preference in their direction of emergence (Figure 3B). In contrast, we found that LR placement was significantly influenced by the presence of localized $CuFe_4O_4Zn$ patches in a concentration-specific manner (Figure 3B); More LRs were placed in the direction of nutrient cores at 1 µM $CuFe_4O_4Zn$ concentration (Figure 3B). This concentration is comparable to concentrations measured in the bulk soil samples from the field (Figure 2E−G). In contrast, cores enriched in 100 µM $CuFe_4O_4Zn$ elicited an avoidance response, resulting in more LRs growing in the direction away from the cue (Figure 3B). $CuFe_4O_4Zn$-enriched cores also induced changes in LR length in a concentration-specific manner; the length of the first emerging LR was highest for seedlings supplied with 1 µM $CuFe_4O_4Zn$ cores (Figure 3C). In addition, aboveground biomass was found to be higher in the presence of 1 µM $CuFe_4O_4Zn$ cores (Figure S1A) and positively correlated with the length of the first emerging lateral root (Figure S1B).

To further explore the extent of LR growth responses, individual micronutrient cues were applied to targeted LR tips and changes to their angle of growth relative to the application point of the cue were monitored over a 24 h period (Figure 3E). The localized application of $CuFe_4O_4Zn$ to targeted LR tips induced directed growth towards the location of the applied cue (Figure 3F). This response was specific to salts containing Cu^{2+} and Fe^{3+} (Figure 3G). For all cues tested, observed orientation changes to LR tips also corresponded with a growth reduction once the roots had reached the micronutrient patch (Figure 3H). Together these results show that *N. attenuata* responds to the presence of localized micronutrient patches by directional root placement and directed growth (also termed chemotropism).

Micronutrient-induced LR bending occurs in an ethylene- and jasmonic acid-dependent manner.

To explore the role that hormone biosynthesis and perception play in controlling micronutrient-induced root bending, we measured the trajectory angle of LR tip growth in transgenic *N. attenuata* plants which ectopically express or are silenced in ethylene- (*sETR* and *irACO*) or jasmonate (JA) signaling related genes (*irAOC*, *irCOI1*, *irJAR4/6*). In comparison to EV control plants, JA-biosynthesis impaired irAOC plants failed to direct root tip growth toward the localized application of $CuFe_4O_4Zn$ (Figure 4), suggesting that jasmonates are required for root chemotropism in *N. attenuata*. In contrast, no clear influence of JAR4/6 and COI1 silencing was observed, pointing to a root chemotropic response that is likely independent of JA-Ile biosynthesis/perception. The chemotropic response was also absent in ethylene-deficient irACO plants, while ethylene-receptor silenced sETR lines showed an intermediate phenotype, indicating that ET-signaling is required for these plant response.

DISCUSSION

A combination of field and laboratory-based assays were used to evaluate the capacity of *N. attenuata* to detect and direct root growth towards localized nutrient patches in its native environment. The analysis of bulk soil surrounding LRs revealed a strong relationship between lateral root placement and soil micronutrient concentration, particularly the abundance of copper, iron and zinc. Apart from serving functions in soil anchorage, the elongation and proliferation of LRs are thought to serve as a means to increasing the volume of soil that can be explored by a root system. In nature, low availability of Fe, Cu, and Zn may be a major limiting factor of plant growth because they form insoluble ferric oxide complexes in soil (Viets 1962). The role that LRs play in accruing limiting nutrients from the soil may vary between mobile and immobile solutes. This was recently demonstrated for *Oryza sativa*, where the LRs play an important role in acquiring less mobile elements such as P, Mn, Zn, Cu and As, but have relatively smaller effects on the acquisition of mobile elements such as N, S and K. (Liu et al. 2013). In our experiments, the application of localized micronutrient salts such as copper and iron to LR tips led to a chemotropic response in laboratory assays. Thus, for *N. attenuata*, it is likely that the supply of Fe, Zn, and Cu induces foraging by LR to actively scavenge for immobile micronutrients complexes.

Our experiments show that localized micronutrient patches are capable of inducing changes to LR placement and growth in *N. attenuata* in a nutrient- and concentration-specific manner in nature, suggesting that LRs rely on both the identity and the size of the nutrient signal. This result is consistent with observations in *Sedum alfredii*, which is known to exhibit strong root growth responses for Zn, by allocating 90% of its root biomass to Zn-enriched zones (Liu et al. 2010). Similarly, in *A. thaliana*, Fe-limitation promotes LR elongation in zones where Fe is highly concentrated (Giehl et al. 2012). This process depends on the hormone auxin and its influx transporter, AUX1; when the LR-defective *arf7arf19* mutants were challenged by restricting Fe availability to a horizontal patch, plants developed symptoms of Fe deficiency in leaves while LRs of wild type plants retained the ability to acquire Fe and maintain normal shoot development (Giehl et al. 2012). Together, these phenotypes emphasize the importance of LRs for the effective acquisition of nutrients, especially less mobile micronutrients, through spatial exploitation for localized patches.

An increase in the horizontal soil exploration by LR is also thought to make a major contribution to the ability of a plant to outcompete neighboring plants when exploiting the same soil niche. For example, the fitness of the *A. thaliana* auxin mutant *axr4* with a reduced number of LRs, was found to be significantly compromised when competing with wild type plants for immobile phosphorous, but not mobile nitrate (Fitter et al. 2002). Whether the morphology of the *N. attenuata* root system serves a similar dual function in detecting conspecific neighbors and acquiring limiting nutrients remains to be determined; however, it is possible that similar effects may be observed for this post-fire annual species which mass-germinates from long-lived seed banks following fires and, in doing, must face strong intraspecific competition within a patchy micronutrient environment.

The application of localized micronutrient cues to targeted LRs demonstrated that fine-scale changes to LR growth might also be associated with foraging behavior. Directed root growth towards a localized micronutrient patch has, to our best knowledge, not been demonstrated before. Whether such growth responses are also stimulated in late-emerging LRs (closer to the primary root tip) in addition to early-emerging LRs remains unknown in *N. attenuata* and may be an area to test in future experiments. Root tips are able to sense the local and internal concentrations of nutrients to adjust growth and developmental processes and optimize the exploratory capacity of the root system (Ruiz Herrera et al. 2015). While the LR bending response observed is analogous to a root gravitropic response, this phenotype is interesting since gravitropic responses are typically suppressed in LRs relative to primary roots (Tian et al. 2014).

This enables the root system to expand radially and reduces competition for the same soil niches among LRs and their parental root, as well as among LRs themselves. The angle of LR growth is thought to be at least partially under genetic control due to tropic responses as was demonstrated for *A. thaliana* and *O. sativa* accessions that display variations in LR angle (Mullen and Hangarter 2003; Iyer-Pascuzzi et al. 2010). This has been attributed to differences in an intrinsically programmed angle gravitropic set-point angle in LR (Digby and Firn 2002) and endogenous hormone signals (Tian et al. 2014).

From the distinct growth dynamics observed in response to different micronutrient cues we inferred that there might be specific hormonal regulators mediating this process. Using available transgenic *N. attenuata* lines, we found that in contrast to wild type EV plants, jasmonate biosynthesis-deficient irAOC and ethylene-deficient irACO plants were impaired in micronutrient-induced LR chemotropism. While these hormones have well-established roles in controlled root developmental processes, these results suggest that they also play additional roles in root tropic growth responses. The phytohormone auxin is well-known to mediate root tropic responses including gravitropism (Feraru et al. 2015). Whether hormonal crosstalk between the jasmonate (JA) and ethylene pathways translates information regarding local nutrient environment into downstream changes in LR growth, and whether it is mediated by auxin signaling remains to be evaluated. Studies suggest that ethylene may regulate root gravitropism by controlling differential cell elongation either in parallel with auxin or as a regulator of the auxin-mediated signaling pathways (Buer et al. 2006; Edelmann and Roth 2006). This hypothesis is also supported by recent evidence demonstrating that the highly JA-responsive ethylene response factor 109 (ERF109) acts as an important point of convergence between JA and auxin signaling pathways by integrating JA signaling into auxin biosynthesis to regulate LR formation in *A. thaliana* (Cai et al. 2014). JA signaling has been linked directly to auxin homeostasis through the modulation of YUCCA8 and YUCCA9 gene expression (Hentrich et al. 2013). Jasmonates have also been reported to regulate auxin transport through effects on the distribution of the auxin exporter PIN-FORMED 2 (Sun et al. 2011). In addition, transcription levels of several genes involved in auxin biosynthesis are up-regulated by methyl jasmonate and mediate JA-induced auxin accumulation and transport during lateral root formation (Sun et al. 2009). Despite these findings, the molecular mechanisms underlying interactions between JA and auxins are far from being well understood. Future work may aim to unravel both the molecular mechanisms that mediate jasmonate- and ethylene-auxin crosstalk and the function of these within *N. attenuata* root systems.

MATERIALS AND METHODS

Characterization of root system architecture in natural populations

To describe the architecture of the root systems of wild *Nicotiana attenuata* plants in nature, twenty *N. attenuata* plants growing in a dry riverbed adjacent to the Lytle Ranch Preserve (St. George, UT, USA) were carefully excavated in the summer of 2011. The sandy riverbed allowed us to excavate intact root systems, which were laid out on the sand and photographed. As a comparison, twelve plants were grown in sand in the glasshouse as described (Machado et al. 2013), and their roots were similarly excavated, washed, and laid out on a flat surface to be photographed.

Lateral root placement and soil nutrient heterogeneity

To investigate whether root placement depends on naturally occurring nutrient heterogeneity in the soil, soil samples were collected from a native *N. attenuata* population located at 37°12.933'N and 113°48.132'W and 1077 m altitude on 8 June 2013. Bulk soil (approx. 30g) samples were harvested at 3 distances from the main stem (5 cm, 15 cm and 25 cm). Samples were taken at each distance along all LRs located within the top 5 cm of excavated soil (on average 2 roots per plant), as well as from one transect per plant originating from the main stem and extending in a direction in which no lateral roots were growing (Figure 2A). Soil elemental analyses were performed by a certified laboratory (Utah State University Analytical Laboratories, Logan, UT, USA). Phosphorus and potassium were extracted by the Olsen $NaHCO_3$ method and quantified by atomic absorption spectrometry (Thermo Electron Solar S2 AA). Ammonia and nitrate were extracted in 2N KCl. Total carbon and nitrogen was determined by combustion methods (LECO TruSpec C/N). The concentration of the micronutrients Fe, Zn, Cu, and Mn were determined by inductively-coupled plasma spectrophotometry (Thermo Electron iCAP-ICP) using $CaCl_2$/DTPA extraction procedures. Principle component analysis was used to visualize the overall effect on the different nutrient concentrations due to sampling location (root or control transect) and distance from the primary root. Data were analyzed in R using FactoMineR (Team 2014).

Plant material and growth conditions in laboratory experiments

Seeds of the 31st generation of an inbred line of *N. attenuata* originally collected from Utah, USA, were used as the wild-type genotype in all experiments, unless otherwise stated. Seeds were first surface sterilized and incubated with 1:50 (v/v) diluted liquid smoke (House of Herbs, Passaic, NY, USA) and 0.1 M gibberellic acid (GA_3). Individual seeds were germinated on 12 x

12 cm vertically-positioned plates sealed with Parafilm M (Pechiney Plastic Packaging; Chicago, U.S.A.) in a randomized design within a growth cabinet (Percival Scientific, Inc; Perry, U.S.A.) under 24-26°C and 16:8h light:dark (Krügel et al. 2002).

Root foraging assays

To investigate changes in root architecture in response to micronutrient deficiency, wild-type *N. attenuata* plants were grown on nutrient agar consisting of modified low-strength Gamborg's B5 medium (0.5g· L^{-1} GB5; Gamborg 196# (Gamborg et al. 1968)) using Duchefa B (Phytoagar, P1003; Duchefa Biochemie, Netherlands). To create a concentration gradient and promote the movement of salts between the enriched cores and the surrounding media, Duchefa B Phytoagar was specially chosen as it is known to contain low levels of Fe, Zn, Cu relative to other commercially available brands (Gruber et al. 2013). To remove trace amount of Fe arising from inevitable contamination by nutrient salts in the agar, this growth media was also supplemented with 75 μM ferrozine [3- (2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulfonate] (Serva, Germany), an iron chelator previously shown to render traces of iron unavailable (Jain et al. 2009). Lateral root placement preferences and growth were monitored in the presence of localized micronutrient patches by supplying a 1 mL (1 cm diameter) agar core enriched in 0.01 μM, 0.1 μM, 1 μM, 10 μM, or 100 μM $CuFe_4O_4Zn$. Cores were placed 3 cm away from a single, centrally located seed at the time of germination. Low-strength GB5 cores served as control. After 14 d of incubation, root systems were scanned using an Epson Expression 10000XL scanner (Seiko Epson Corp; Nagano, Japan) in color at 300 dpi resolution and lit from the back. Image contrast was adjusted using Adobe Photoshop CS6 (Adobe Systems Inc.) to allow for better quantification of roots. Lateral root length and density were measured in scanned images using line drawing tools in ImageJ (Rasband 1997). Following the scans, leaf and root biomass measurements were also recorded.

Chemotropism assays

To test whether *N. attenuata* roots orient towards nutrient cues, seedlings were germinated on modified low concentration GB5 medium as described above. After 14 d, the first emerging lateral root of each plant was randomly assigned to micronutrient ($CuFe_4O_4Zn$) or control (H_2O) treatment, marked with an "X" 1 cm below the growing root tip, and scanned as described above. Scans were taken and angles were measured immediately prior to application of a 10 μL volume of 100 μM $CuFe_4O_4Zn$ or H_2O cues (T_0), and compared to LR angles measured 24 h later (T_1, Figure 3E). The highest concentration of $CuFe_4O_4Zn$ was chosen due to the two orders of magnitude difference between the volume of the agar core in the experiment above and the

volume of the cue described here (1 mL versus 10 µL). Root angles were measured from the marked location of the cue, the origin of the emerging LR from the primary root, and the growing LR tip using the ImageJ angle tool (Figure 3E). The angle of deviation of targeted LR tips was calculated by subtracting the observed angle at T_1 from the angle measured at T_0. In doing so, a positive value indicates LR growth *toward* a given cue. A value of zero indicates no change in growth angle, while a negative value denotes growth away from the cue. To test for elemental specificity of a directed LR growth response, this experiment was also carried out by applying 10 µL of 100 µM $Fe_2(SO_4)_3$, $FeSO_4$, $CuSO_4$, or $ZnSO_4$ as micronutrient cues. All foraging and chemotropism experiments were performed at least twice and yielded similar results.

Phytohormone regulation of LR chemotropism

Interactions among plant hormones form a complex network to regulate developmental processes within the roots (Wolters and Jürgens 2009). Ethylene is a strong controller of root development, and it has been suggested that it is involved in the increase of lateral root development in nutrient-rich soil patches (selective root placement) (Visser et al. 1997; Clark et al. 1999; Visser et al. 2008). Jasmonates (Wasternack and Hause 2013; Wasternack 2014) are known to affect root growth and development in various ways and were therefore chosen as good candidates to gain initial insight into the regulation of LR growth responses toward localized micronutrient patches. To explore the role that the biosynthesis and perception of these hormones play in controlling nutrient-induced root chemotropism, we made use of transgenic *N. attenuata* plants which over-express or are silenced in ethylene- (*sETR* and *irACO*) (von Dahl et al. 2007) or jasmonate-related (*irAOC*, *irCOI1*, *irJAR4/6*) genes (Paschold et al. 2007; Wang et al. 2008; Kallenbach et al. 2012). Previously characterized phenotypes of each of these lines are summarized in Table S1. Growth of root tips relative to micronutrient patches (10 µL of 100 µM $CuFe_4O_4Zn$) or water control cues was quantified in each transgenic line and compared to root responses of an empty vector (EV, A-03-9-1) control line. The angle of deviation of LR tips relative to the micronutrient cues was quantified as described above.

Statistical analyses

Unless otherwise notes, statistical tests were performed using SigmaPlot version 12.5 (Systat Software, Inc.). Normality and equality of variance were verified using Shapiro–Wilk and Levene's tests, respectively. Data were analyzed using analysis of variance (ANOVA) followed by pairwise comparisons made using the Holm-Sidak Method.

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AUTHOR CONTRIBUTIONS

A.P.F and M.E. conceived the original research plans; A.P.F., C.C.M.A., and R.A.R.M. performed and analyzed lab experiments; A.P.F., M.E., R.A.R.M., D.K. and I.T.B. performed and analyzed field experiments; A.P.F and M.E. wrote the first draft of the manuscript with contributions from all the authors; All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Figure S1. Seedling biomass in the presence of localized micronutrient cores

(A) Localized micronutrient patches induce changes to total (ANOVA P=0.02) and leaf biomass (P=0.02), but not root biomass (P=0.32). Cores were placed 3 cm from a single, centrally located seed at the time of germination. See Figure 3A for experimental petri dish set-up. Root (filled bars) and leaf (hatched bars) biomass measurements were recorded two weeks after germination. Control plants (magenta bars) were supplied with 0.5 g· L^{-1} GB5 cores. Blue bars are $CuFe_4O_4Zn$-supplied roots. Letters denote significant differences in total biomass between micronutrient concentrations (P<0.05) in *post hoc* comparisons. Bars are mean ±*SE* (N=12-14). **(B)** The length of the first emerging lateral root (LR) positively correlates with leaf biomass. Results of Pearson's correlation are shown.

Table S1. Characteristics of the transgenic *Nicotiana attenuata* lines used

ir: inverted repeat silencing, s: ectopic expression, JA-Ile: jasmonoyl-l-isoleucine.

FIGURE LEGENDS

Figure 1. Root phenotypes of glasshouse-grown and wild *Nicotiana attenuata*

(A) Relative to the roots of transplanted seedlings grown in the glasshouse (left panel), roots excavated from natural populations display a single primary root, and few, well-developed lateral roots (right panel, lateral roots denoted with arrows). Lateral roots remain within shallow layers of soil (first 5 cm) and may extend horizontally for up to 40 cm. **(B)** Histogram showing the number of lateral roots per plant excavated from natural populations. Results are from 20 plants. **(C)** Lateral root proliferation and formation of first order lateral roots (LR) within dense soil substrates are also commonly observed under natural conditions.

Figure 2. Lateral root placement within a natural population of *Nicotiana a ttenuata* corresponds to high micronutrient concentrations

(A) Principal component analysis was used to visualize the overall effect on the different nutrient concentrations due to transect type (root versus control) and sampling distance (5, 15, 25 cm). An individual factors map was generated from the PCA with PC1 versus PC2 and correlation coefficients obtained for correlations between the variation within individual nutrient concentrations and the variation within each PC. Vectors display the relationship between lateral root placement and individual nutrients. Concentrations of macro- **(B−D)** and micronutrients **(E−H)** were determined in bulk soil samples surrounding lateral roots located within the top 5 cm of soil. Soil samples containing the highest Zn, Cu and Fe concentrations (0.49 mg/kg, 0.67 mg/kg. and 7.05 mg/kg, respectively) corresponded to visible increases in proliferation and the formation of first order lateral roots. See Figure 1C for representative examples of such phenotypes. Results of two-way ANOVA are shown with asterisks indicating a significant difference between soils collected from control versus root transects (**, $P<0.05$, *** $P<0.001$ Holm-Sidak *post hoc* comparison). All points are the mean ±*SE* (N=4).

Figure 3. Localized micronutrient patches induce root foraging and chemotropic behavior in seedlings

(A) Experimental petri dish set-up to examine root placement and foraging phenotypes in the presence of localized micronutrient cores. Cores (highlighted by dashed outlines) were placed 3 cm from a single, centrally located seed at the time of germination. Scale bars = 1 cm. **(B)** $CuFe_4O_4Zn$-enriched cores induce preferential placement of the first emerging LR in a concentration-specific manner. Asterisks indicate significant placement preferences by χ^2 analysis (*** $P<0.001$; N=12-14). Control roots (filled bars) were supplied with 0.5 g· L^{-1} GB5 cores. Hatched bars are $CuFe_4O_4Zn$-supplied roots. **(C)** $CuFe_4O_4Zn$ patches induce changes in LR growth in a concentration-specific manner. **(D)** Changes in growth are independent of changes in LR density. The length of the first emerging LR extending toward $CuFe_4O_4Zn$-enriched or control (0.5 g· L^{-1} GB5) cores was measured using line drawing tools in ImageJ. LR density was calculated as the total number of visible LR divided by the primary root length. Bars in (**C**) and (**D**) represent the mean ±*SE* (N=12−14). **(E)** $CuFe_4O_4Zn$-induced deviation in LR angle was measured using the ImageJ angle tool immediately prior to the localized application of a 10 µL volume of 100 µM $CuFe_4O_4Zn$ salt cue located 1 cm from a targeted LR top (T_0) and compared to LR angles measured 24 h later (T_1). Root tip angle was determined from the growing root tip

relative to the applied cue (X, red). The white hatched arrow denotes the angle arm between the base of the seedling and the nutrient cue (X), which does not change between T_0 and T_1. The red hatched arrow indicates the arm of the angle that shifts according to the change in root tip growth. The degree of deviation was determined by subtracting the angle value observed at T_1 from the angle at T_0. A positive value indicates root growth toward the micronutrient cue. A value of zero indicates no change in growth angle, while a negative value denotes growth away from the cue. Scale bars = 1 cm. **(F)** Localized $CuFe_4O_4Zn$ induces directed LR growth toward the site of application. Bars are mean ±*SE* (N=26). **(G)** Cu^{2+} and Fe^{3+}-containing sulfate salts induce the strongest directed LR growth response. **(H)** $CuFe_4O_4Zn$-induced changes to LR angle are independent of changes to LR growth. Results of One-way ANOVA are shown with asterisks indicating significance in Holm-Sidak *post hoc* comparison relative to control (****P*<0.001; ** *P*<0.05). Bars in (**G**) and (**H**) are mean ±*SE* (N=16–19).

Figure 4. Micronutrient-induced lateral root chemotropism is mediated by ET biosynthesis and perception and JA biosynthesis, but not JA-Ile biosynthesis or JA perception

Root tip angles of petri dish grown seedlings were measured in EV control and transgenic lines (ir: inverted repeat silencing, s: ectopic expression, JA-Ile: jasmonoyl-l-isoleucine) using the ImageJ angle tool at the time of application of the 10 µL 100 µM $CuFe_4O_4Zn$ cue and 24 h later (as in Figure 3E). A positive angle value reflects directed root tip growth toward the salt cue. A value of zero indicates no change in growth angle, while a negative value denotes growth away from the cue. Values are shown relative to average change in root tip angle induced by the control cue (H_2O). Results of one-way ANOVA are shown with asterisks indicating significant differences between $CuFe_4O_4Zn$- and H_2O–supplied roots. All bars represent the mean ±*SE* (N=4–6). ** *P*<0.05 Holm-Sidak *post hoc* comparisons between transgenic lines (filled) and EV control (hatched).

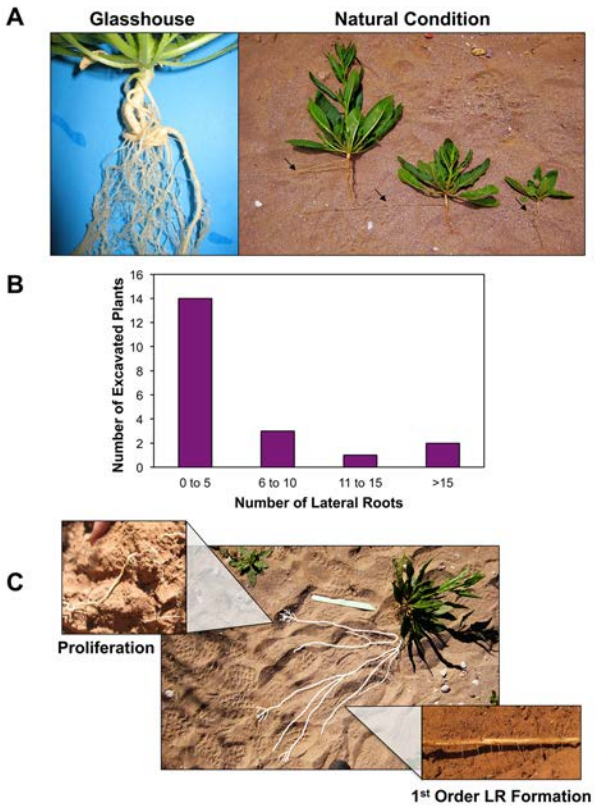

Figure 1

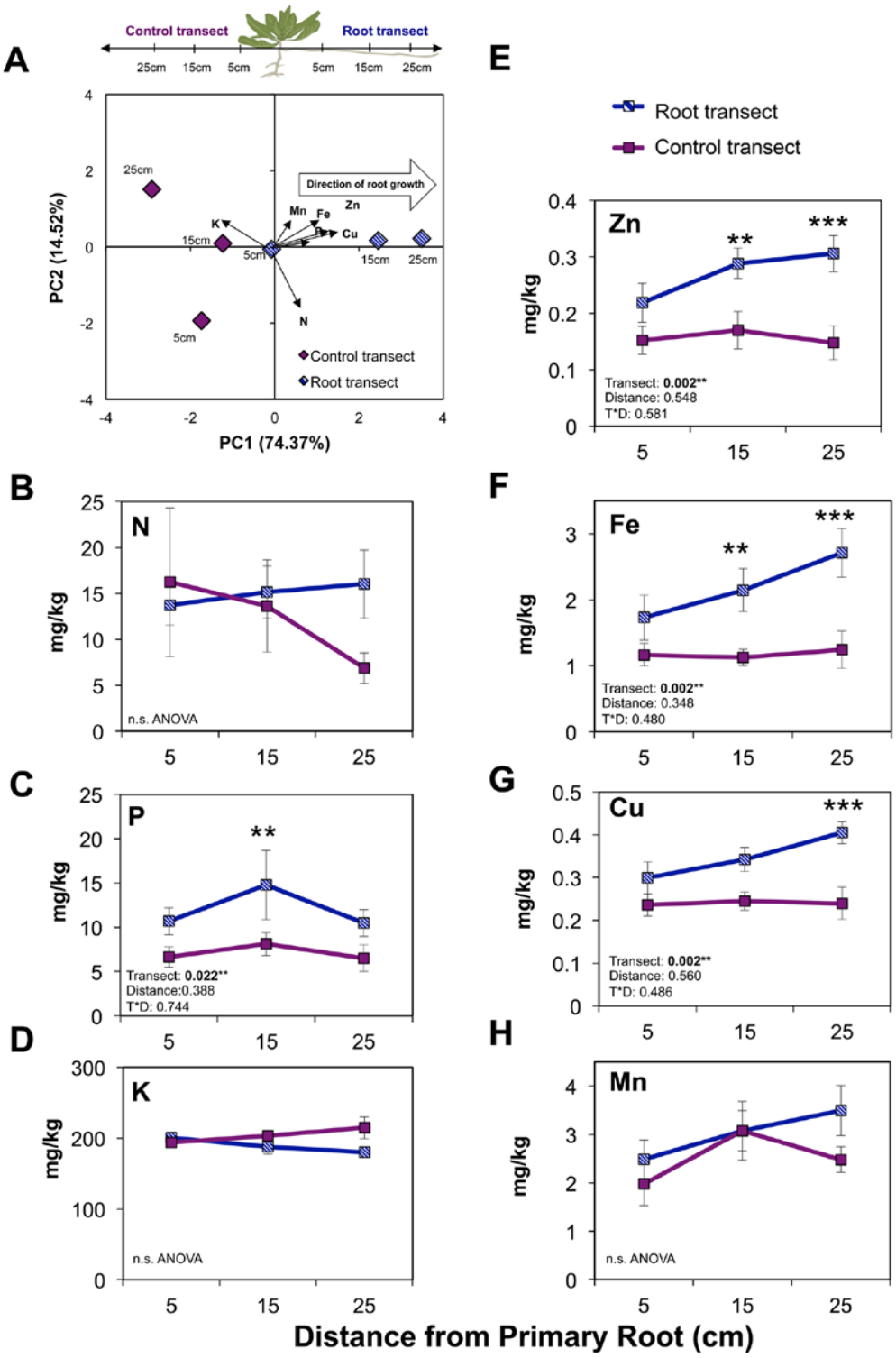

Figure 2

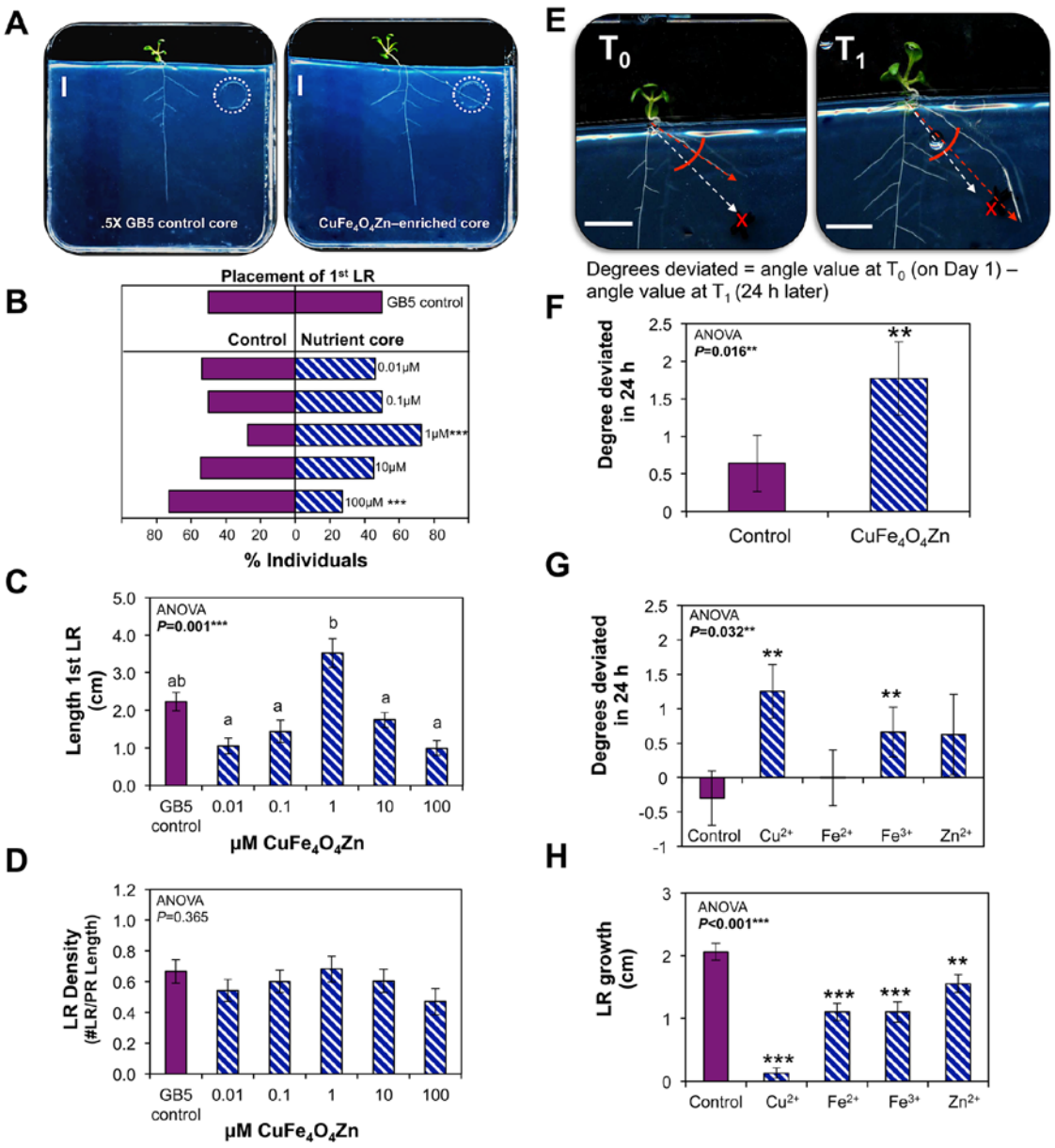

Figure 3

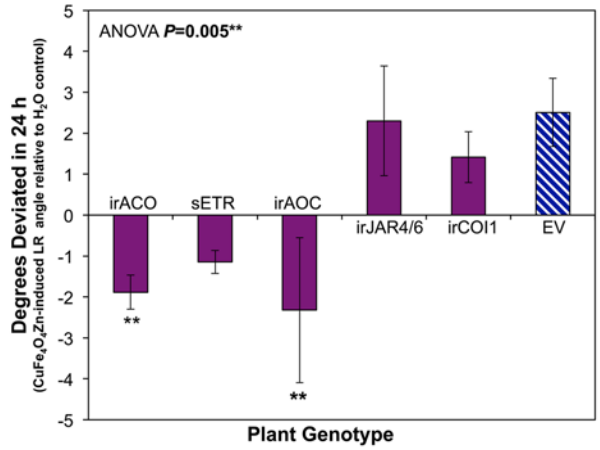

Figure 4